

Original article

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Urine tropenol ester levels in workers handling tiotropium bromide synthesis: implications for exposure prevention and biomonitoring

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Tropenol ester is a highly toxic anticholinergic substance and an intermediate used in industrial production of the bronchodilator tiotropium bromide. The aim of this study was to systematically test workers involved in its production for tropenol ester in urine to identify any exposure pathways and define additional preventive measures. Twelve workers performing tasks involving potential exposure to tropenol ester were repeatedly monitored at the end of each production cycle. Medical exams revealed no symptoms of acute poisoning with tropenol ester, but biological monitoring of urine showed 36 positive findings in 79 samples, with tropenol ester concentrations ranging between the detection limit of 54 pg/mL and 2160 pg/mL. We managed to establish the cause of only one positive finding, which was a hole in a protective glove, whereas the rest most likely occurred due to human error. Because of this, the plant decided to modify the production process by replacing tropenol ester with a safer intermediate. While it is the safest course of action, there where it cannot be taken, biological monitoring can be very helpful in raising awareness about exposure to toxic substances, including the new ones that have not been studied for their adverse potential.

KEY WORDS: anticholinergic agents; intermediates; prevention; scopine ester; workplace analysis

Industrial innovations – whether they involve new technologies or new chemical substances – inevitably raise issues about occupational safety during their development and production (1–3) despite sophisticated safety measures and intensive training of employees.

One such chemical substance is tropenol ester [di-(2-thienyl)glycolic acid tropenol ester] (4), a precursor of the bronchodilator tiotropium bromide (5). Tropenol ester is readily absorbed through the skin, binds to muscarinic acetylcholine receptors, and has an exceptionally high receptor affinity (4). In 2012, we reported a case of tropenol ester poisoning with severe anticholinergic symptoms that lasted unusually long – two weeks. Fortunately, they were completely reversible. Earlier, in 1994, we reported work-related poisoning with another anticholinergic intermediate used in the synthesis of tiotropium bromide, i.e. scopine ester [scopine di-(2-thienyl)glycolate] (6, 7). After the acute anticholinergic symptoms had subsided, symptoms of a newly developed encephalopathy became apparent and did not improve over the next eight years of follow-up. Repeated thorough medical examinations did not provide evidence of any non-occupational cause of encephalopathy (personal observation). In contrast to FDA-approved tiotropium bromide, both of these intermediates used in its

synthesis can quickly pass the blood-brain barrier as tertiary amines, have high receptor activity, and are readily absorbed through the skin, which explains their severe toxic action (4, 6).

The case of tropenol ester poisoning came as a surprise to all involved and triggered a detailed review of all precautionary measures in the company. Apparently, all precautions had been taken to prevent occupational exposure. Production was not continuous but was carried out in cycles lasting two weeks. The equipment used for production was set up for each cycle and cleaned after production was completed. The only anticholinergic intermediates used were tropenol ester and scopine ester. The workers were potentially exposed to tropenol ester throughout the shift, especially when shovelling dry powder from/into reaction vessels, taking samples for analysis, cleaning equipment, and taking off protective clothing. These operations cumulatively lasted about one hour per shift. Scopine ester was used as a solution in a closed system. Each worker basically carried out all activities. There was no rotation schedule. During the last production cycle, only particularly experienced employees shovelled tropenol ester. Employees wore chemical-resistant boots, protective suits, gloves, and full masks with P3 filters for all activities. Protective gloves were made of butyl or nitrile. The manufacturer had tested their permeability with a powder of tropenol ester and found none over the 480 minutes of the test. The wearing times during operation

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were considerably shorter. Gloves were changed after each activity involving exposure to tropenol ester. In addition, new gloves were used at the beginning of each shift. The scalp and the skin on the neck was completely covered. Employees wore overshoes over their boots. The overshoes were attached to the protective suit with adhesive tape to prevent contamination of the employees' work boots. After each operation involving potential exposure, the protective clothing and gloves were first decontaminated and only then removed and disposed of. There were changing rooms with separate storage options for work and street clothes. The employees showered after work. The concentration of tropenol ester in the air was never measured because measurement would have had no practical purpose for occupational safety. Exposure could only occur during cleaning of the equipment and removal of protective clothing. Yet, no systematic deficits were found. The most likely cause of acute poisoning was human error (4).

Since human error can never be prevented with certainty and both intermediate products are highly toxic, intensified efforts were made to improve occupational safety by introducing urine biomonitoring for tropenol ester as the least inconvenient sampling method for the workers. The detection of an intermediate product in workers' urine was intended as a red flag that would launch an investigation into possible exposure pathways. To implement this practical approach as quickly as possible, we skipped a full validation (8) and focused on detection rather than reliable quantification of internal exposure. What follows is a report on our findings and steps taken to improve the implemented occupational safety measures, including biological monitoring.

PARTICIPANTS AND METHODS

Participants and medical advice

The company physician informed the workers about the required industrial hygiene and possible symptoms of poisoning with an anticholinergic compound in detail. In the event of symptoms or potential exposure, the workers were instructed to immediately refer to the company medical office, which was staffed around the clock. They were also invited to take additional preventive measures, i.e. medical check-ups and biological monitoring and all 12 of them responding willingly, aware of the risks of tropenol ester poisoning. Good communication is essential in this respect (9). At the time of the first check-up, the median age of the 12 men was 26 years (range 21–43). One worker left the department after the second production cycle for personal reasons not related to potential exposure. He was not replaced.

Biological monitoring

For practical reasons, the workers gave a urine sample at the end of each production cycle. Perceived exposure to an anticholinergic compound was not reported. Therefore, no additional inquiries about perceived exposure were necessary.

Urine samples were also taken from 12 participants unexposed to anticholinergics for method validation. The samples were collected in vessels containing 10 mL of citric acid (1 mol/L) for stabilisation. Ten-millilitre aliquots of the acidified samples were stored at -20 °C until analysis. Tropenol ester in urine was determined with a modified liquid chromatography – tandem-mass spectrometry (HPLC-MS/MS) method for rat plasma developed by the pharmaceutical manufacturer. After pH adjustment with sodium hydroxide, the urine samples were extracted by liquid-liquid extraction with methyl tert-butyl ether and the extracts analysed for two ion transitions: 362.1>122.0 and 362.1>140.1. The chromatograph was calibrated by external calibration with internal standard correction. Each sample was analysed in duplicate, quantifying concentrations for both ion transitions. Urinary analyte concentrations were reported as mean values of the duplicate analyses and concentration calculations. No exhaustive validation data are available for the described analytical method, but repeatability and accuracy were ensured by repeated analyses of quality control samples spiked with three known concentrations of tropenol ester [low (272 pg/mL), medium (1090 pg/mL), and high (5440 pg/mL)] in each analytical series (n=6 for each concentration). The coefficients of variation as a measure of inter assay-repeatability were 12.9 % at low concentration and ion transition 362.1>140.1 but <7 % for all other concentrations and transitions. Relative recovery as a measure of the method's accuracy was between 96.5 % and 103.6 % for all concentration levels and both ion transitions. The method's lower limit of quantification (LLOQ) was 54 pg per mL of urine. The detection limit was also 54 pg/mL. Since the main goal of our investigation was to detect internal exposure, we made no further adjustment of the urinary tropenol ester concentrations (e.g., by means of the specific weight or the creatinine concentration).

RESULTS

Symptoms

None of the employees described any physical discomforts at any point during any of the examinations. None of them experienced any symptoms or clinical signs suggesting adverse effects of exposure to an anticholinergic compound such as dry mouth, thirst, fixed and dilated pupils, flushed face, fever, hot, dry, and red skin, tachycardia, impaired speech, blurred vision, swallowing, or impaired motor activity or coordination (4, 10).

Exposure

None of the employees reported any perceived exposure to anticholinergic compounds. Table 1 shows the urine concentrations of tropenol ester for each participant after completion of each production cycle. The highest concentration found was 2160 pg/mL. In 36 of 79 measurements, the concentrations were above the detection limit of 54 pg/mL. Positive urine samples triggered in-depth investigation. The first two authors thoroughly checked all personal protective equipment and found a hole in the protective glove of the participant no. 12, who was measured 1340 pg of tropenol ester per mL of urine at the end of the first production cycle. No other causes were found that could explain other positive samples.

DISCUSSION

With additional precautionary measures taken in our study, not even a mild poisoning was established. However, we were surprised by positive urine findings for tropenol ester in almost half of the samples, and only in one case it could reasonably be associated with a hole in a protective glove. In this context, it is worth noting that tropenol ester was processed as powder. No solvents were used that could have damaged protective gloves or clothing.

In retrospect, it is not possible to associate individual urine concentrations with specific activities. That no concrete cause of internal exposure was established for the 35 positive samples is unsatisfactory from the point of view of occupational safety, regardless of the absence of poisoning symptoms in any of the workers. We can only speculate about possible exposure routes. Since tropenol ester is easily absorbed by the skin, and respiratory protection was continuously worn, the most likely route

was skin. Even a minor negligence in occupational hygiene can lead to dermal absorption (4, 11). This points to a human error, which may have occurred during removal of protective clothing, but there was no evidence to confirm the assumption. After the first production cycle, tropenol ester was detected in 11 of 12 samples, and after the last cycle only in 1 of 11 (Table 1). Perhaps the awareness of the results of biological monitoring contributed to better personal occupational hygiene. However, experience shows that necessary protective measures are not always followed, even if the risks are known (12).

Interpretation of our findings would benefit greatly from the knowledge about the toxicokinetics of tropenol ester (13), but there is none. This is the major limitation of our investigation, as, without this information, the choice of sample (urine) and the timing of collection relied on practical reasons. Furthermore, there are no limit values for tropenol ester in urine or other biological material that can be used to evaluate the measured concentrations. What we do know, however, is that all of our participants were symptom-free, even if their samples showed traces of tropenol ester. Our earlier case report (4) gives an indication of the level that can be associated with poisoning (4). Namely, we later measured the concentration of tropenol ester in the remaining urine sample of the poisoned patient taken between two and five hours after reported exposure. It was 0.5 mg tropenol ester/L urine, that is, more than 200 times higher than the highest concentration measured in this study (2160 pg/mL, Table 1).

Clearly, tropenol ester concentrations established in our study did not cause acute toxicity in healthy individuals. However, persons who are more sensitive due to illness, e.g. narrow-angle glaucoma, could be more susceptible to anticholinergics, and would have to avoid every risk of exposure. The company physician had made sure that none

Table 1 Concentrations of tropenol ester in urine (pg/mL) and percentage of positive samples per production cycle

Production cycle	1	2	3	4	5	6	7
Worker							
1	2160	500	75	250	<LOD	<LOD	<LOD
2	605	130	<LOD	<LOD	<LOD	<LOD	<LOD
3	365	170	<LOD	<LOD	83	395	140
4	60	70	<LOD	115	<LOD	<LOD	<LOD
5	1390	170	125	150	415	145	<LOD
6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	100	<LOD	*	*	*	*	*
8	300	<LOD	420	<LOD	<LOD	<LOD	<LOD
9	180	75	<LOD	710	<LOD	120	<LOD
10	180	<LOD	365	1530	<LOD	<LOD	<LOD
11	210	200	<LOD	<LOD	<LOD	<LOD	<LOD
12	1340	350	<LOD	175	<LOD	250	<LOD
All subjects, % ≥ LOD	91.7	66.7	36.4	54.5	18.2	36.4	9.1

Samples were taken at the end of each production cycle (1–7); * worker left production; LOD – limit of detection, 54 pg/mL

of the employees in his care had any of these illnesses. We, however, did not look for possible immunomodulatory effects. Acetylcholine receptors including the muscarinic ones are also expressed by epithelial, mesothelial, and immune cells (14, 15). Recent findings suggest that the immune function can be modulated by manipulating immune-cell cholinergic activity with specific agonists and antagonists (15). Perhaps a future research should investigate whether occupational exposure to tropenol ester could actually have an immunomodulatory effect.

In the meanwhile, tropenol and scopine ester were excluded from the production process as intermediates to prevent any exposure and thus acute poisoning and unknown immunomodulatory effects. Judging by internet search results showing that a number of plants worldwide still use scopine and tropenol ester to produce tiotropium bromide, it is quite conceivable that their workers are at risk of exposure. In this case, prevention of the kind described above is indicated.

In conclusion, although tropenol ester is now rarely used, the basic principle of exposure prevention and biomonitoring can be applied to other newly developed substances, their precursors, and by-products of synthesis reactions. Even at the development stage and without established risk thresholds, these substances or their intermediate products should be investigated for toxicity and preventive measures considered based on this knowledge. Experience has shown that individual and systemic errors can be detected with biological monitoring. Positive samples can be a starting point for investigating exposure pathways that were previously unknown (9, 11, 16). Subsequently, targeted occupational safety measures can help to minimise the risk of poisoning. However, since human error cannot be excluded, the best course of action is to replace a hazardous working substance with a non-toxic or at least a less dangerous substance.

Conflicts of interest

Axel Muttray and Bernd Roßbach declare no conflict of interest. Michael Schneider is the company physician of the plant where the production took place.

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Razine tropenol estera u mokraći radnika na sintezi tiotropijeva bromida – implikacije za sprječavanje izloženosti i biomonitoring

Tropenol ester je iznimno toksičan antikolinergik, koji se rabi za sintezu bronhodilatatora tiotropijeva bromida. Cilj je ovog ispitivanja bio sustavno testirati radnike koji njime barataju u proizvodnji na tropenol ester u mokraći ne bi li se ustanovili putevi izloženosti i poboljšale preventivne mjere. Ispitivanje je obuhvatilo 12 radnika kroz 12 proizvodnih ciklusa, a uzorkovanje se provodilo na kraju svakog ciklusa. Liječničkim se pregledom nisu otkrili nikakvi simptomi akutnog otrovanja tropenol esterom, ali se biomonitoringom otkrilo 36 pozitivnih uzoraka od njih 79. Razine tropenol estera u pozitivnim uzorcima kretale su se od granice detekcije (54 pg/mL) do 2160 pg/mL. Međutim, samo smo za jedan pozitivan nalaz uspjeli utvrditi uzrok. Radilo se o rupi u zaštitnoj rukavici. Za ostale pozitivne uzorke pretpostavljamo da upućuju na ljudsku pogrešku prilikom skidanja ili čišćenja opreme. Zbog nemogućnosti da utvrdimo uzroke izloženosti, tvornica je promijenila proizvodni proces i tropenol ester zamijenila sigurnijim međuproizvodom. Iako je to najsigurniji način za sprječavanje izloženosti opasnim tvarima u proizvodnji, tamo gdje to nije moguće, biomonitoring može biti itekako koristan za podizanje svijesti o izloženosti, osobito kod novih tvari čije štetno djelovanje za zdravlje još nije istraženo.

KLJUČNE RIJEČI: analiza radnog mjesta; antikolinergici; međuproizvodi; prevencija; skopin ester